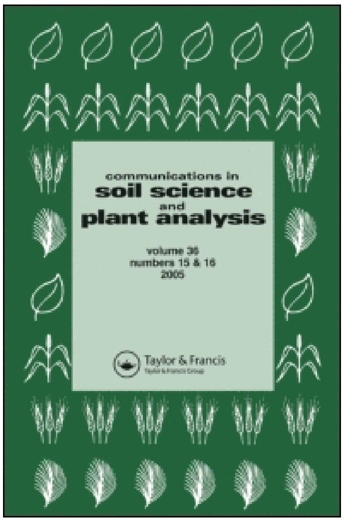

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Enzyme Activities in Soil Influenced by Levels of Applied Sulfur and Phosphorus

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Enzyme Activities in Soil Influenced by Levels of Applied Sulfur and Phosphorus

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Abstract: Soil biochemical properties are influenced by management practices and the type of plant cover, and such changes affect the levels of enzyme activities in soil. In turn, enzymes influence the nutrient supply in soil and growth and mineral composition of the plants. Acidic infertile upland soils are relatively colder in crop-growing season than low land soils and require application of sulfur (S) and phosphorus (P) to improve crop production. Field experiments were carried out on Gilpin silt loam (Typic Hapludult at elevation of 908 m) soil with initial application of four levels of S (0, 16.8, 33.6, and 67.2 kg S ha^{-1}) and three levels of P (22.4, 89.6, and 358.4 kg P ha^{-1}). Acid phosphatase (AP), arylsulfatase (AS) and urease (UR) activities were determined 3 years after the initial fertilizer application. Increasing levels of soil-applied S decreased enzyme activities, but the highest level of applied S (67.2 kg S ha^{-1}) in fact stimulated higher enzyme activities. Increasing levels of soil-applied P reduced AP activities significantly and resulted in reducing trends in AS and UR activities. Overall, the enzyme activities were reduced significantly with increasing soil depth. Activities of all three enzymes were significantly correlated with soil moisture content, total carbon (C) and nitrogen (N), S, and organic sulfur. Findings of this research are helpful in designing management systems that could improve the production potentials of acid soils.

Keywords: Acid soils, enzymes, phosphatase, sulfatase, urease

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INTRODUCTION

Soils of the Appalachian region are acidic and infertile, and addition of P fertilizer and lime are essential to enhance P supply and reduce soil acidity to improve production potentials. Surface horizons of these soils generally have a high organic matter content. Enzymes in soil play a dominant role in, transformation of organically bound nutrients into inorganic plant-available forms (Skujins 1976; Speir and Ross 1978; Stevenson 1986). Enzyme activities in soil are known to serve as an indicator of soil health and to mediate and serve as a catalyst for soil functions such as organic matter decomposition, release of inorganic nutrients for plant growth, N_2 fixation, detoxification of xenobiotics, nitrification, and denitrification (Dick 1997; Nadiaye et al. 2000). In infertile acidic hill land soils under cool temperature, application of S and P are needed to improve plant production. Application of P and S improves productivity, photosynthesis, N_2 fixation, and cold tolerance in legumes (Douglas and Risk 1981; Whitehead 1982). Hern et al. (1988) reported that Ladino white clover recovered faster during cool temperatures and assimilated more S and N with high application of S in a low-fertility acidic soil. In soil, a large portion of the P and S is in organic forms (Speir and Ross 1978; Dalal 1978; Tabatabai and Bremner 1970b). In a surface soil, organic P constitutes between 15 and 80% of total P (Speir and Ross 1978; Dalal 1978), and phosphatase enzymes are believed to play a major role in transformation of organic P into mineral P (Speir and Ross 1978; Tabatabai 1994). In soil, organic S comprises 0–90% of the total S (Speir and Ross 1978; Stevenson 1986), and sulfatase enzymes are believed to play a major role in the mineralization of organically bound S (Skujins 1976; Speir and Ross 1978). Levels of enzyme activities in different types of soil have been related to various soil physical, chemical, and biochemical properties (Skujins 1976; Speir and Ross 1978; Baligar et al. 1988a; Bremner and Mulvaney 1978). Lime, fertilizer, and organic and inorganic amendments added to soil are known to influence the level of enzyme activities (Speir and Ross 1978; Bremner and Mulvaney 1978). In acid soil, addition of lime generally increases sulfatase activities and decreases phosphatase activities; however, addition of phosphate decreases activities of phosphatase, sulfatase, and urease (Haynes and Swift 1988). These soils are generally low in N, and urea is a major source of fertilizer N. Urease catalyzes the hydrolysis of urea to carbon dioxide and ammonia. High-urease activities might result in excess hydrolysis of urea and subsequently lead to loss of ammonia by volatilization or lead to rapid nitrification and nitrate N might be lost by leaching (Skujins 1976; Bremner and Mulvaney 1978). Soil management practices and nature of plant cover influences soil physical, chemical and biochemical properties, and such changes are known to influence the levels of enzyme activities (Speir and Ross 1978; Bremner and Mulvaney 1978; Dick 1984). Type of vegetation influences the nature of organic matter entering the soil, which has a significant effect on variability in enzyme activities (Speir and Ross, 1978; Stevenson, 1986). The objectives of the experiment reported in this article are 1) to evaluate the effects of S and P applied to acidic upland soil on levels of acid phosphatase (AP), arylsulfatase (AS), and urease (UR) activities under white clover cover and 2) to assess the relationship between enzyme activities and selected soil properties.

MATERIALS AND METHODS

Experimental Site and Treatments

Field experiment was carried out on acidic Gilpin silt loam (Typic Hapludult at elevation of 908 m) soil with pH_{H_2O} 5.0 in southern West Virginia. In this study, four levels of S (0, 16.8, 33.6, and 67.2 kg S ha^{-1}) and three levels of P (22.4, 89.6, and 358.4 kg P ha^{-1}) were applied in combination. Sulfur was applied as $MgSO_4$ and P was applied as Ca $(H_2PO_4)_2 \cdot H_2O$. All the treatments received a blanket application of 100 kg K ha^{-1} as KCl and 2240 kg of lime ha^{-1} as $CaCO_3$. These amendments were surface applied on a roto-tilled experimental area. All the mineral fertilizers were applied at the start of the experiment. Roundup was applied 2 weeks prior to seeding to eliminate unwanted plant species. Ladino white clover (*Trifolium repens* L.) seeds were planted with a power-till seeder at the rate of 17 kg ha^{-1}. A complete randomized block design was used, and all treatment combinations were replicated four times.

Soil Sampling and Analysis

Three years after the initial application of mineral fertilizers and five seasonal harvest of white clover, two replications were selected at random to collect soil samples. Two 30-cm^2 areas per plot were selected at random for soil sampling from two replications. Crop residues were removed, and soil was sampled at 0 to 7.5- and 7.5- to 30.0-cm depth. Soil samples were passed through a 1-mm sieve, and a composite sample of soil was placed in a plastic bag, sealed, and transported to the laboratory in an ice chest. Soil samples were stored at 4°C until assayed for enzyme activities. The moisture content of soil samples was determined by drying soil for 48 h at 105°C. Soil samples were extracted by Bray-1, and P and S in the extract were determined by ICP. Total C and N were determined by Leco CHN 600 analyzer (Leco Corporation, St. Joseph, MI). Organic S was computed by taking the difference between total S determined by Leco SC 132 analyzer and extractable S from Bray-1 extractant. Soil at the experimental site had a pH-H_2O (1 : 1) of 6.2 with total C and N of 39 and 3 g kg^{-1} and extractable P and S of 10 and 25 mg kg^{-1}. The total S and organic S were

287 and 260 mg kg^{-1}, respectively. Addition of S from 0 to 67.2 kg ha^{-1} increased extractable S from 25 to 35 mg kg^{-1}, and addition of P from 22.4 to 358.4 kg ha^{-1} increased Bray-1 P from 10 to 153 mg kg^{-1} (data not shown).

Enzyme Assay

Enzymes were assayed on soil samples stored at 4°C. The acid phosphatase was assayed according to method suggested by Eivazi and Tabatabai (1977) and Tabatabai and Bremner (1969) using p-nitrophenyl phosphate as substrate. Arylsulfatase was assayed according to Tabatabai (1994) and Tabatabai and Bremner (1970a) using p-nitrophenyl sulfate as substrate. The urease activities were assayed by using urea as substrate and extracting the ammonia released by 2 *M* KCl and ammonia detected by Technicon auto analyzer II (Tabatabai 1994 Tabatabai and Bremner 1972). Enzyme activities were expressed on an oven dry soil weight basis. Duncan's multiple-range test was used to evaluate statistical significant differences in each enzyme activities at varying S and P levels and soil depth and their interactions. Linear correlation coefficient analysis (SAS program) was used to relate enzyme activities to soil physical and chemical properties.

RESULTS AND DISCUSSION

Acid Phosphatase (AP)

Increasing levels of applied S from 0 to 33.6 kg S ha^{-1} reduced AP activities; however, at 67.2 kg S ha^{-1} application, AP activities increased significantly (Tables 1 and 2). Increasing levels of applied P reduced AP activities significantly. Linear, inverse, and no relationships between AP activities and soil inorganic P content have been reported (Speir and Ross 1978; Baligar et al. 1988a, 1988b; Haynes and Swift 1988). The AP activities were significantly related to total and organic S, and the relationship between AP activities and inorganic S content of soil was negative and insignificant (Table 3). Speir (1984) reported a positive relationship between AP activity and adsorbed S in Tongan soils and a negative relationship in Cook Island soils. Tongan soils had relatively less adsorbed S than Cook Island soils. The AP activities declined significantly with increasing soil depth (Tables 1 and 2). Decreased root and microbial activities and reduced organic P, C, and N content of soil have been attributed to reduced AP activities at lower soil depth (Speir and Ross 1978; Baligar et al. 1988a, 1988b). The AP activities were significantly correlated with original soil moisture content. Baligar et al. (1988a) reported that AP activities in 14 hill land soils were significantly correlated with soil moisture content and percent water-filled pores. Significant positive relationships between AP activities and soil C and N content were found (Table 3).

Table 1. Long-term effect of applied S and P on enzyme activities under white clover at different depth[a]

Sulfur (kg/ha)	P (kg/ha)	Acid phosphatase		Arylsulfatase		Urease	
		SH[b]	SSH	SH	SSH	SH	SSH
0.0	22.5	897	545	378	198	187	104
	89.6	738	568	402	211	188	178
	358.4	744	510	400	204	154	108
16.8	22.5	874	640	401	257	180	117
	89.6	685	458	396	165	194	98
	358.4	695	469	404	206	153	126
33.6	22.5	618	535	332	188	153	100
	89.6	690	506	276	162	75	75
	358.4	662	508	280	142	115	66
67.2	22.5	805	602	330	164	190	126
	89.6	861	550	260	216	136	101
	358.4	800	554	390	173	172	138

[a]Acid phosphatase and arylsulfatase activities are μg p-nitrophenol released g soil^{-1} h^{-1} and urease activities as μg of NH_3 released g soil^{-1} 2 h^{-1}.

[b]SH, surface horizon (0–7.5 cm); SSH, subsurface horizon (7.5–30.0 cm).

Such relations are well documented in several other types of soils (Speir and Ross 1978; Baligar et al. 1988a, 1988b; Speir 1984; Baligar et al. 1999). Level of AP activities observed in this study are comparable with the AP activities reported in many other types of soils (Speir and Ross 1978; Baligar et al. 1988a, 1988b; Speir 1984; Baligar et al. 1999).

Arylsulfatase (AS)

Increasing levels of soil-applied S reduced AS activities, but added P had no significant effect on level of AS activities (Tables 1 and 2). The AS activities declined significantly with increasing soil depth. A reduction in AS activities with increasing soil depth in various types of soil is well documented, and such a decline is often related to reduction in soil organic C and moisture content (Tabatabai and Bremner 1970b; Baligar and Wright 1991). The AS activities were positively and significantly correlated with soil moisture and C and N content, however, the relationship with extractable P was nonsignificant (Table 3). A significant positive relationship between AS activities and soil C has been well documented (Tabatabai and Bremner 1970b; Speir 1984; Baligar et al. 1999). Baligar and Wright (1991) reported a low level of AS activities in soils that contained relatively low amounts of C, N, and organic P and S. This indicated that the organic matter in soil plays a fundamental

Table 2. Enzyme activities as influenced by levels of S, P, soil depth[a]

Variables	Acid phosphatase	Arylsulfatase	Urease
Sulfur, kg/ha			
0.0	667ab[b]	299a	143a
16.2	639b	305a	145a
33.6	586c	230b	113b
67.2	695a	255a	145a
Phosphorus, kg/ha			
22.4	691a	281a	145a
89.6	632b	261a	135a
358.4	617b	274a	129a
Depth, cm			
0.0–7.5	756a	354a	166a
7.6–30.0	538b	190b	107b
Analysis of variance			
Sulfur (S)	**[c]	*	NS
Phosphorus (P)	**	NS	NS
Depth (D)	**	**	**
S × P	**	NS	NS
S × D	*	NS	NS
P × D	NS	NS	NS
S × P × D	NS	NS	NS

[a]Acid phosphatase and arylsulfatase activities are μg p-nitrophenol released g soil^{-1} h^{-1} and ureas activity as μg of NH_3 released g soil^{-1} 2 h^{-1}.

[b]Means within a column for each of the variables not followed by the same letter differ at the 0.05 level of probability by DMR test.

[c]*, **significant at the 0.05 and 0.01 level of probability, respectively.

NS, not significant.

role in establishing the level of AS activity. Such a relation between AS activity and soil organic matter suggested the possibility for AS to be present as humic-protein complexes that protect it from microbial-decomposition (Ladd and Butler 1975). In the current study, relations between AS activities and total and organic S were highly positive and significant. However, the relations between AS activities and extractable S were negative and significant, and the relations between AS activities and extractable P were positive and nonsignificant. Speir (1984) reported positive and negative relationships between AS activities and extractable P in Cook Island and Tongan soils. Baligar and Wright (1991) with Appalachian soils and Baligar et al. (1999) with Brazilian soils reported positive relationships between AS activities and organic and extractable P and S content of soils. Earlier studies have reported that AS activities have no relation to total S and that they are only weakly related to inorganic S (Speir and Ross 1978; Tabatabai and Bremner 1970a). Positive and

Table 3. Correlation coefficient (r) between activities of various enzymes and enzyme activities and soil properties

Variables	Acid phosphatase	Arylsulfatase	Urease
Soil moist	0.51**[a]	0.56**	0.35*
C	0.76**	0.88**	0.77**
N	0.74**	0.85**	0.74**
P	0.20NS	0.29NS	0.14NS
Total-S	0.77**	0.77**	0.77**
Organic-S	0.78**	0.78**	0.77**
Inorganic-S	−0.30*	−0.30*	−0.18*
Acid phosphatase	1.00	0.73**	0.71**
Arylsulfatase	—	1.00	0.80**

[a]*, ** significant at the 0.05 and 0.01 level of probability, respectively.
NS, not significant.

negative (Speir 1984) relations between AS activities and adsorbed S have been reported. High degree of positive correlations between AS activities and soil moisture content confirmed the earlier findings of Baligar and Wright (1991) and Spier (1984). The magnitude of AS activities observed in the current study are comparable with AS activities reported in other soil types (Tabatabai and Bremner 1970a; Speir 1984; Baligar et al. 1999; Baligar and Wright 1991).

Urease (UR)

Increasing levels of applied S and P tend to decrease UR activities. A significant reduction in UR activities was observed with increasing soil depth (Tables 1 and 2). In many soils, a decline in UR activities with soil depth has been attributed to reduction in soil organic C content (Bremner and Mulvaney 1978; Baligar et al. 1991; Dalal, 1975; Zantua et al. 1977). Highly significant and positive relationships were observed between UR activities and organic C and total N content of soil (Table 3). In many other types of soil, a high degree of correlation exists between UR activities and soil organic C and N (Speir 1984; Baligar et al. 1991; Dalal 1975). Such a positive relationship between UR activities and soil C content might be due to a higher level of microbial biomass and greater stabilization of extracellular UR by humic molecules (Burns 1978). Pancholy and Rice (1973), however, found poor correlation between UR activities and organic C content of soil. The significant correlation between all three enzymes and soil N content may be an indirect consequence of significant correlation between soil organic C and N (Baligar et al. 1988a; Baligar and Wright 1991; Baligar et al. 1991). Significant and positive correlations were observed between

UR activities and soil moisture content. Baligar et al. (1991) also reported similar relations between UR activities and soil moisture content in 14 acidic hill land soils. Urease activities were positively related to inorganic P, and total and organic S. Positive and negative relations between UR activities and forms of P and S in other types of soils have been reported (Speir 1984; Baligar et al. 1991, 1999). The level of UR activities observed is comparable with UR activities in various types of soils (Speir 1984; Dalal 1975; Pancholy and Rice 1973).

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